OBSERVATIONS ON SOME PHASES OF THE LIFE CYCLE OF *ICHTHYOPHTHIRIUS MULTIFILIIS* FOUQUET, 1876, A CILIATE PROTOZOAN PARASITE OF FRESH-WATER FISH.

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(Plates vi. and vii.)

INTRODUCTION.

The material for this paper was obtained by the author while investigating, in his capacity as Biologist to the Victorian Freshwater Research Committee, two serious outbreaks of ichthyopthiriasis (White Spot, itch disease or Fleckenkrankheit) at the trout hatcheries of the Ballarat Fish Acclimatisation Society in 1939 and 1940.

The field aspect of the problem has been discussed already in a previous paper (Butcher, 1941); in the present communication certain incidental observations on the life history of the infecting organism will be dealt with.

TECHNIQUE.

The material used in this investigation was obtained from the Ballarat hatcheries during the severe outbreak in the 1939-40 season. Live infected trout were brought to the laboratory and kept in suitable aquaria. I have experienced the same difficulty as have most previous investigators in maintaining the parasite under these artificial conditions. MacLennan (1937), by maintaining aquaria at a constant temperature of approximately 26°C. by means of an electric heating element controlled by a thermostat, has succeeded in maintaining the infection; these facilities unfortunately were not available to me. However, on two occasions, using carp, a complete life cycle was obtained under these adverse conditions, and this will be reported on fully later in this paper.

Observations on the free-living parasite after leaving the fish were made on numerous individuals pipetted out from the aquaria into petri dishes or other suitable receptacles. Under these conditions the encystment and post-encystment phases can readily be observed on the living material. In addition, I have examined fixed material, using Corrosive Acetic, Carnoy, Bouin, etc., as fixatives and various stains, including Methyl Green and Acid Fuchsin, Ehrlich's, Delafield's, and Heidenhain's Haematoxylin, etc.

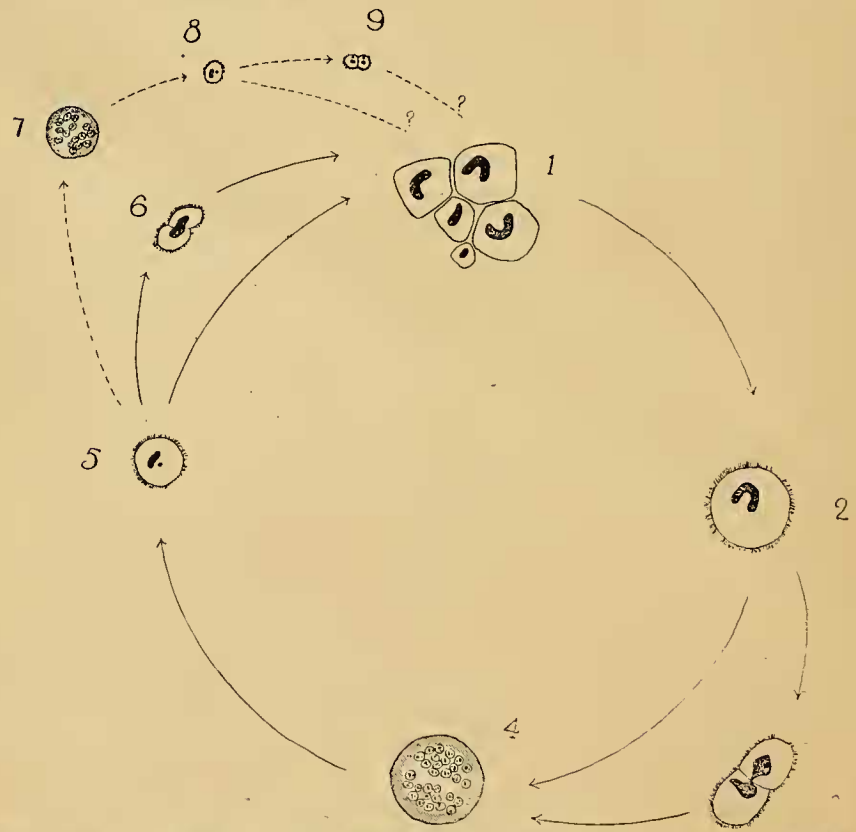
The parasite on the fish was examined in the living condition and on formalin fixed material after paraffin imbedding and staining with Delafield's Haematoxylin.

OUTLINE OF THE LIFE CYCLE.

A preliminary outline of the life history of the ciliate will facilitate the subsequent description.

The complete life cycle is shown in text-fig. 1. The adult organism (1) emerges from the pustule and becomes the free-swimming precystic form (2) with a diameter of approximately 420 micron, although diameters of up to just under 1 mm. have been recorded. This form may either directly

encyst (4) or may first undergo a single division (3). By repeated binary fission within the cyst the minute postcystic forms (5) arise and these may either directly infect another fish or may first undergo a single division (6). There is no certain evidence of a sexual phase; see Haas (1933). The terms precystic and postcystic are used in the sense in which I defined them in my earlier paper. The minute postcystic forms are variously spoken of by other authors as swarm spores, ciliospores or simply spores. In addition to the life cycle which takes place when fish are available for infection, another, and perhaps abnormal, cycle of events may ensue in which the postcystic form may re-encyst (7) and undergo further reproduction in this condition. This accessory cycle is indicated by dotted lines in text-fig. 1. Whether the forms which thus arise are capable of infecting fish I could not determine.



Text fig. 1.—Diagrammatic representation of the life cycle of the parasite. (Broken arrows represent phases which occur only if no host is available.) 1.—Organisms in pustule on host. 2.—Precystic form. 3.—Division in precystic form. 4.—Cyst. 5.—Postcystic form. 6.—Division in postcystic form. 7.—Second cyst. 8.—Second postcystic form. 9.—Division in second postcystic form.

OBSERVATIONS.

1.—Free Living Phases.

(a) Division in the precystic stage.

Usually the parasite (fig. 1) after leaving the fish, proceeds to encyst; but occasionally, as Stiles (1893) has already observed, a single division may precede encystment (figs. 2-5); these two halves I have observed to encyst, but their further development was not followed. Like MacLennan (1937) I have never encountered an example of the complete multiplication of the ciliate in the free-swimming condition as described by Minchin (1922); in all instances the multiplication is completed, in my experience, within the cyst wall.

Not uncommonly I have found that one of the products of this division of the precystic form is a peculiarly shaped organism having a nipple-shaped projection at one end (fig. 3).

(b) Encystment.

At a time ranging from 3 to about 25 hours after liberation from the fish encystment of the parasite takes place. As already stated a single fission may precede encystment; but if more divisions take place, then, as MacLennan (1937) has also observed, the ciliates do not encyst but disintegrate.

It would seem, in agreement with MacLennan (1937), that the parasites must have attained to a certain degree of maturity on the fish for encystment to take place; for if liberated artificially from the pustules then encystment rarely occurs.

I have on several occasions been able to observe the actual process of encystment. First the ciliates drop to the bottom of the vessel, their movement becoming gradually slower, then the beat of the cilia practically ceases and locomotion by the action of cilia gives way to a type of amoeboid movement. A single pseudopodium developes and the flow of protoplasm can be followed as in an amoeba. At this period the cilia are still present but are not sufficiently active to move the parasite. Finally all movement ceases, the cilia disappear, and the thick wall of the cyst is formed. The cyst wall is surprisingly thick; this will be readily seen from fig. 6, which is a camera lucida drawing of a living cyst.

Cyst formation takes place on any suitable submerged object. There is, however, no certain evidence that encystment normally occurs on the bodies of fishes. Zacharias (1893) states that he frequently found encysted individuals on a young fish, and Roth (1908) also recorded the presence of such cysts on the skin of living fishes. I have myself never seen anything to confirm this. According to MacLennan (1937) it is quite a common occurrence to find encystment on the semi-detached epithelium of a dead fish.

(c) The cyst.

Owing to the great variation in size of the precystic forms there is a large range of variation in the size of the cysts themselves, and, in consequence, in the number of postcystic forms arising from the cysts.

Within the cysts repeated binary fission of the organism now ensues. I

have observed the process in numerous fixed and stained preparations (Methyl Green and Acid Fuchsin was a satisfactory double stain for cysts). There is considerable difficulty in obtaining the cysts intact, for they are firmly attached to the substratum. This difficulty can be overcome satisfactorily, without injury to the delicate cyst, by drawing a fine glass thread under it.

Division does not always proceed with any regularity within the cyst. Sometimes a complete partition may develop across the cyst, division in the two halves proceeding independently, and the postcystic forms leaving from their respective sections. Stages in the division of the cyst content are shown in Figs. 7-10, fig. 8 being the two-celled stage, fig. 9 the four-celled stage and fig. 10 the sixteen-celled stage. Fig. 7 shows an example of the extreme irregularity of division which is sometimes encountered, four of the division products having lagged much behind the remainder.

In my experience the number of fission products liberated from the cysts never exceeds a few hundred. This agrees roughly with Doflein's (1909) observation; other authors, Fouquet (1876) and Prytherch (1923) have observed them in much greater number. Prytherch quotes cases where they were liberated up to 2,200 in number.

(d) Postcystic form.

After 8 to 24 hours (at an average temperature of 17°C.) the young postcystic forms begin to emerge from the cyst. As they work their way through the gelatinous cyst wall they become elongate but soon revert to a more or less spherical shape (figs. 11-12). These postcystic forms measure about 75 micron in diameter. I could not with certainty recognise the presence of a mouth; MacLennan (1935) states, however, that he has been able to detect one.

Fouquet (1876), Pearson (1932) and MacLennan (1935) all agree that the young postcystic forms remain elongate long after leaving the cyst, but this has never been my experience.

(e) Division of the postcystic form.

The postcystic forms are now ready to infect another fish. Nevertheless at times the postcystic form may divide once again (figs. 15-16) before reinfecting a fish; this occurs, indeed, fairly commonly, but to my knowledge has been noted by only one other worker, Buschkiel having recorded it in 1911.

(f) Survival of the postcystic form in absence of the host.

In a number of experiments the young postcystic forms were isolated in petri dishes, without the opportunity to gain access to a fish. Under these conditions a phase in the life cycle was revealed, which has not, to my knowledge, been hitherto observed. Within 24 hours, at least fifteen of the free-swimming forms had again encysted, and within the cysts division of the ciliates recommenced. Owing to the minute size of the organisms a limit is necessarily set to their further reproduction; actually cases ranging from 2 to a maximum of 16 new individuals were observed within the cyst. By the end of the second day these second postcystic forms (fig. 17) were swimming around, having been liberated naturally from the cysts. They continued to swim around for about 24 hours and

some had even begun to divide as free-swimming ciliates (figs. 18-19). The diameter of the second postcystic forms prior to this division was about 24 micron. At this period unfortunately, following on the introduction of water from a different source into the petri dishes, the culture became contaminated with another small ciliate, of approximately the same size but of different shape, and the observations had to be discontinued. Whether these second postcystic forms are themselves capable of infecting fish I have not determined.

The appearance of these minute second postcystic forms as seen in fixed and stained preparations is shown in figs. 17-19. The preparations were obtained by mixing the culture fluid with egg albumen which was spread on to a coverslip and fixed in the ordinary way with Corrosive Acetic and stained with Delafield's Haematoxylin.

2.—Parasite on the Fish.

I have already alluded above to the difficulty which I and most other observers have had of bringing about natural infection of fishes with *Ichthyophthirius multifiliis* in aquaria. Nevertheless on two occasions, using carp, such infections did take place, thereby completing a life history under experimental conditions. These two cases have been included in Table I. It will be observed that under these conditions at a temperature of about 17°C. the complete life cycle has a duration of 13-16 days. It is probable that this furnishes an explanation to the periodicity of the epidemics that have been observed among trout at the Ballarat hatcheries. There were in one season four recorded outbreaks of Ichthyophthiriasis of varying degrees of severity and these occurred at regular intervals of about a fortnight; namely, in the middle of November, the beginning of December and the middle of December. The next was recorded on the 30th January of the following year. Presumably there was a less virulent and therefore undetected outbreak during the middle of January. The temperature range throughout these months was 14.5 to 18°C.

(a) Mode of entry.

In order to observe the passage of the postcystic ciliate into the host I placed a detached caudal fin from a recently killed healthy fish into a petri dish to which were added numerous free-swimming postcystic forms. Very soon the parasites settled on the fin and began to rotate with considerable speed. After a period of approximately 40 minutes the organisms had by this means burrowed into the epithelium and could no longer be detached by violent agitation. This confirms essentially the observations of Neresheimer (1908), Buschkiel (1911), Haas (1933) and MacLennan (1935 a. & b.). Haas and MacLennan were actually able to detect a large solid hyaline knob or perforatorium at the anterior end of the ciliate, free of cilia, which the organism seems to use as a wedge to force an entrance between the cells.

(b) Site of infection.

This has been described as occurring in the epithelium over the entire head, body, fins and gills, as well as in that of the mouth, opercula and around the eyes. Wolf (1938) found in sections through rainbow trout that the parasites were almost entirely beneath, not in, the epidermis, sometimes even partly beneath the scales.

TABLE 1.

Duration of phases of the life cycle under laboratory conditions.

Life History	Exptal. Host	Av. Temp. °C.	Precystic	Cystic	Postcystic	Spots First Noticeable	On Fish	Total Length	Remarks
(1)	Small carp	16.0	16½-19¼ hours						
(2)	„ „	17.2	8-11 hours	15-24 hours					The period on the host in these life cycles refers to the reinfected fish and not the original host which was obtained already infected.
(3)	„ „	16.8	3-25 hours (Mostly 3-11 hours)	8-16 hours	Sev. hours	After 4 days	12-15 days	13-16 days	
(4)	„ „	16.0	3-6 hours	12-21 hours	Sev. hours	After 3 days	11 days	13 days (approx.)	

In my own work sections through a complete brown trout fry and through portions of six others, some two thousand sections in all were examined. Parasites were found in all the positions described above and also in others. The majority of the parasites were in the epidermis itself (see figs. 24, 25), and many others were in positions as described by Wolf (1938). Some were in contact with the connective tissue layers (see fig. 26), others in contact with the developing scales, and others again in the actual pockets of the developing scales (see fig. 26). These deeply embedded parasites were not in the majority, as in the case described above by Wolf. In one case a parasite was observed in a lymph space.

The most unexpected position in which a parasite was found was in the actual body cavity of its host (see fig. 27), the parasite being in the posterior end, in the region of the cloaca. As it was thought that this might be a isolated case, serial sections were made through the cloacal region of six more infected fish. Parasites were found in the body cavity in three more cases; two parasites were found in the cloaca of the fourth and only two had no parasites in this region. It would, therefore, appear that the presence of parasites in this position is a regular occurrence. It is not difficult to visualise the passage of the parasite from the cloaca through to the body cavity, as the wall of this chamber is quite thin. In no case had the parasite moved far along the body cavity.

(c) The pustule.

The pustules vary greatly in size, being sometimes as much as a millimetre or more in diameter. Cases containing as many as three or four parasites per pustule have frequently been deemed worthy of comment in the literature; it has therefore been a matter of considerable surprise to find in the Ballarat outbreak that individual pustules sometimes contained over fifty organisms. These cases are, of course, extreme, but even an observed average of six (based on one hundred counts) is unusually high. Probably this is related to the extraordinary severity of the infection in the hatcheries; a quart of water taken at random from one of the ponds contained many hundreds of the free-swimming parasites. Within the pustules the parasites lie closely compressed and often considerably distorted. This will be seen in figs. 13 and 14 (fig. 14 represents only a fragment from a single large pustule).

As Fouquet (1876) and, following him, many other observers have recorded, the parasites undergo slow rotation within the pustules. This is due to the action of cilia at their surface. (Cilia are usually difficult to see in fixed preparations and were not visible in the organisms from which figs. 14 and 15 were drawn).

Several explanations have been advanced for the presence of more than one parasite within a single pustule. It may be due (1) to union of adjacent pustules (Prytherch, 1923); (2) to the simultaneous entrance of more than one individual at one point, Buschkiel (1911) having observed a congregation of as many as ten individuals at the point of entrance; (3) to various ciliates using already formed entrances and passage ways under the epidermis (Buschkiel, 1936). There is no critical evidence for deciding between the three suggestions.

From time to time there have been reports of the actual division of the parasite within the pustule—Stiles (1893), Prytherch (1923), Roughley

(1933) and Suzuki (1935). Neither Doflein (1909), nor Haas (1933) nor MacLennan (1935a) could find any evidence for this. In my own experience, based on the examination of many hundreds of pustules, there has been no evidence whatsoever for such fission. Roth (1908) and Buschkiel (1911) both state that division takes place on the fish—not in the pustule, but in the slime covering the fish.

3.—Occurrence of a Mouth and the Supposed Occurrence of an Anus.

There has been much discussion as to the position and shape of a mouth in *Ichthyophthirius multifiliis*. In his original description, Fouquet (1876) described it as a small prominence which has a circular opening with a divided edge "like a stamping machine" and occurs at the extreme anterior end of the body; according to Zacharias (1893) it is ventral in position in the anterior third; Kerbert (1884) placed it laterally near the anterior end of the body; Stiles (1893) states that it is situated terminally at the posterior extremity; Guberlet (1933) places it at the anterior end, while Haas (1933) and MacLennan (1935) describe it as sub-terminal in position.

My own observations have been made (1) on the living precystic form viewed on a dark background (figs. 20-21); (2) on sections of the same (fig. 23); (3) on sections through the parasite on the host (fig. 22). In my material the oral opening is situated almost terminally; it is circular and is surrounded by a thickening of the cuticle; it leads into a ciliated gullet. The cilia surrounding the mouth are approximately the same length as those covering the body; those lining the gullet are very much longer. The mouth opening in all forms examined was, however, considerably wider than any I have seen illustrated by the authors above referred to.

A possible explanation for the discrepancies in the accounts of the mouth given by various observers, including myself, relating as they seem to do to one and the same species of *Ichthyophthirius* lies perhaps in the difference of host and of environmental conditions under which the organisms existed.

Bütschli (1883), Doflein (1909) and Wenyon (1926) all record the presence of an anus or cytopyge. Neither Fouquet (1876) or Kerbert (1884) were able to observe one, but Kerbert states that the faeces are expelled from various points on the surface of the organism. Stiles (1894) could observe neither an anus or defecation. I have myself examined large numbers of individuals, both in section and in the living condition, without being able to detect any trace of an anus.

It is noteworthy that MacLennan (1935 b, 1936) has even been able to observe the retention of indigestible material by one of the products of fission after encystment, this faeces-laden form being non-viable.

4.—Effect upon the Fish.

(a) Feeding.

Fouquet (1876), Zacharias (1893) and Prytherch (1923) uphold the view that within its host the ciliate absorbs liquid nutriment directly through the surface of its body. As long ago as 1884, however, Kerbert claimed to have observed the ingestion of pigment cells from the host into the body of the parasite. MacLennan (1935 b.) also speaks of the ingestion of host tissue in the form of small globules 5 micron in diameter derived by

fragmentation of host cells. Pearson (1932), on the other hand, holds that both types of feeding occur.

My own observations leave no doubt as to the ingestion of whole cells, often in large quantity, into the protoplasm of the parasite (figs. 30-31). Most of the ingested cells lie freely in the cytoplasm, although some are contained in food vacuoles (see particularly fig. 31). I have not observed the presence of fragmented cells as described by MacLennan.

Whether in addition fluid nourishment is absorbed into the parasite I have not been able to determine.

(b) Reaction of the skin.

In the extremely heavy infections with which I have worked I have found in all cases a very pronounced thickening of the epidermis—the consequence of a proliferation of its cells. This may be seen by comparing fig. 29 from an uninfected fish and fig. 28 from a heavily infected fish. Since in heavily infected fish the parasites are scattered over the entire body, practically the whole epidermis has undergone this thickening. Even when the parasites are found in the deeper layers of the skin the overlying epidermis shows this thickening. This proliferation does not, however, take place in the epithelium of the gill filaments (fig. 30).

(c) The cause of death of the fish.

No satisfactory explanation has yet been given for the markedly lethal effect of the parasite upon fish. Commonly we find that infection with *Ichthyophthirius multifiliis* is followed by attacks of *Saprolegnia*, and Doflein (1909) regards this secondary infection as the actual cause of death, whilst Roughley (1933) states that these *Saprolegnia* growths upon the gills may have the effect of choking the fish. Nevertheless, in the Ballarat epidemics, when several hundred thousand fish perished, *Saprolegnia* was conspicuously absent. Wolf (1938) also recorded an epidemic with heavy mortality in the complete absence of the fungus. Prytherch (1924) and Roughley (1933) observed red blotches and heavy secretion of slime over the fish; neither of these symptoms again were in evidence in the Ballarat outbreak. It is to be noted that Roughley's observations were made on aquarium fish; Prytherch's on catfish, bass, bream and sunfish, whilst my own observations were on rainbow and brown trout and carp. Stiles (1893) could recognise no gross lesions, death being due presumably to general injury of the epidermis and the enormous amount of slime present on the body and over the gills. MacLennan (1935 a. & b.) also attributes death to epithelial destruction, at least in heavy infections. Yet in my own material there has been little evidence of epidermal destruction, the epidermis showing, on the contrary, a considerable thickening. Wolf (1938) suggests that death may be due to osmotic derangement consequent upon epidermal injury; but there is no evidence, in the form of water-logging of the fish, to support this.

I can only suggest as the most likely hypothesis that the parasite is the source of a toxin which is the cause of death. It is worth noting that, even in the trout, which is a very susceptible species of fish, some individuals are unaffected by the parasites; for in the Ballarat outbreaks there were sometimes up to a dozen healthy fish left in ponds in which several thousand fish had died. These fish could not possibly have escaped the

general infection. Buschkiel (1911) suspected that immunity might arise and carried out an investigation into the matter, but came to no conclusion. Unlike many other hosts, carp appears to possess a natural tolerance for *Ichthyophthirius multifiliis*. It acts as a carrier, and from my knowledge, apart from small ornamental types, is seldom killed.

SUMMARY.

The life cycle of the parasite consists of four major phases; the parasitic phase on the host; the precystic stage; the cystic stage and the postcystic stage.

The life cycle is not, in fact, always so simple, as division may occur in the precystic and postcystic stages. It is further complicated by reencystment of the first postcystic form in the absence of the host.

Detailed periods are given for each phase of the life cycle, and the total time for the complete life history obtained under laboratory conditions (13-16 days) probably furnishes an explanation to the periodicity of the epidemics observed in the Ballarat hatcheries; the temperature range in each case was approximately the same.

The ciliates penetrate the epithelium and other tissues of the host by means of a boring apparatus.

The numerous positions on the host in which the parasite is found are described; of particular note is the discovery of parasites in the posterior end of the body cavity.

The number of organisms found within the pustules varies immensely, being sometimes as many as fifty individuals; possible explanations for this heavy infection are outlined.

The species of fish infected may have some influence on the form of the mouth of *Ichthyophthirius multifiliis* which has been described differently by various authors. A description is given of the mouth in living forms and in sections through the ciliate. There is no anus and defecation does not take place.

The parasites feed on the whole cells which they dislodge.

As a reaction to very heavy infection, proliferation of the cells over the whole of the epidermis occurs, with the exception of that of the gill filaments.

Various suggestions as to the cause of death of the host are discussed and a hypothesis that a toxin is produced is brought forward.

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EXPLANATION OF PLATES.

All drawings made with aid of camera lucida from fixed specimens (except where otherwise stated); cilia not shown except in figs. 1 and 23.

(Plate i.)

Fig. 1. The precystic form of the parasite; note the typical horse shoe-shaped macro-nucleus. X 30.

Figs. 2- 5. Division in the precystic form. All X 30.

Fig. 6. Living cyst; illustrating the thickness of the wall. X 30.

Fig. 7. Cyst; the initial division has been repeated only twice in one portion of the cyst. X 30.

Fig. 8. Cyst; initial division. X 80.

Fig. 9. Cyst; 4-cell stage. X 80.

Fig. 10. Cyst; 16-cell stage. X 80. The author was unable to obtain a series of cysts, in different stages of division, of uniform size. The cysts illustrated in figs. 8-10 have developed from precystic forms differing greatly in size.

Figs. 11-12. Postcystic forms of the parasite. X 90.

Fig. 13. Small pustule on the skin of a fish. X 30.

Fig. 14. Portion of a large pustule on the skin of a fish. Note the variety of forms assumed by the parasites in the restricted space of the pustule. X 30.

Figs. 15-16. Division in the postcystic form. X 80.

Fig. 17. Second postcystic form. X 167.

Figs. 18-19. Division in the second postcystic form. X 167.

In fig. 19 the products of the division have not yet separated. As the mount was made in a thick egg albumen preparation each of the individuals had to be drawn at a different focal distance. The second individual in each case is indicated by the shaded outline.

(Plate ii.)

Figs. 20-21. Living precystic forms of the parasite showing position and form of the mouth; drawn against a dark background.

Fig. 22. Section through a parasite (taken from a fish) illustrating

the form and position of the mouth. The nucleus has been cut through in two places. X 132.

Fig. 23. Section through a free parasite showing the long cilia lining the distinct gullet. X 80.

Fig. 24. Transverse section through portion of an infected fish; parasite imbedded in the epidermis. X 82.

Fig. 25. Transverse section through portion of an infected fish; the parasite is imbedded in the epidermis and epithelial cells, dislodged by the parasite, may be seen in the space of the pustule surrounding the parasite. X 132.

Fig. 26. Transverse section through portion of an infected fish; the parasite is in the pocket of a developing scale and in contact with the connective tissue. X 82.

Fig. 27. Transverse section through an infected fish; the parasite (p) is in the body cavity of its host. X 21.

Figs. 28-29. Transverse sections through portion of an infected and a healthy fish respectively. The epidermis of the infected fish is much thicker than that of the healthy fish as indicated by the line drawn on the left-hand side of each figure. X 82.

Fig. 30. Section through a parasite in a gill filment from an infected fish. Note the single ingested cell within the food vacuole. X 80.

Fig. 31. Section through a parasite taken from an infected fish; five ingested cells may be seen in a food vacuole. X 132.